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ORIGINAL ARTICLE

Influence of Immunogenicity on the Long-Term Efficacy of Infliximab in Crohn's Disease

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ABSTRACT

BACKGROUND

Treatment with infliximab, a chimeric monoclonal IgG1 antibody against tumor necrosis factor, can result in the formation of antibodies against infliximab. We evaluated the clinical significance of these antibodies in patients with Crohn's disease.

METHODS

In a cohort of 125 consecutive patients with Crohn's disease who were treated with infliximab infusions, we evaluated the concentrations of infliximab and of antibodies against infliximab, clinical data, side effects (including infusion reactions), and the use of concomitant medications before and 4, 8, and 12 weeks after each infusion.

RESULTS

A mean of 3.9 infusions (range, 1 to 17) per patient were administered over a mean period of 10 months. Antibodies against infliximab were detected in 61 percent of patients. The presence of concentrations of 8.0 µg per milliliter or greater before an infusion predicted a shorter duration of response (35 days, as compared with 71 days among patients with concentrations of less than 8.0 µg per milliliter; $P < 0.001$) and a higher risk of infusion reactions (relative risk, 2.40; 95 percent confidence interval, 1.65 to 3.66; $P < 0.001$). Infliximab concentrations were significantly lower at four weeks among patients who had had an infusion reaction than among patients who had never had an infusion reaction (median, 1.2 vs. 14.1 µg per milliliter; $P < 0.001$). Patients who had infusion reactions had a median duration of clinical response of 38.5 days, as compared with 65 days among patients who did not have an infusion reaction ($P < 0.001$). Concomitant immunosuppressive therapy was predictive of low titers of antibodies against infliximab ($P < 0.001$) and high concentrations of infliximab four weeks after an infusion ($P < 0.001$).

CONCLUSIONS

The development of antibodies against infliximab is associated with an increased risk of infusion reactions and a reduced duration of response to treatment. Concomitant immunosuppressive therapy reduces the magnitude of the immunogenic response.

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INFLIXIMAB (REMICADE, CENTOCOR), a chimeric monoclonal IgG1 antibody against tumor necrosis factor, has been approved for the treatment of moderate-to-severe Crohn's disease in patients who have an inadequate response to conventional therapy and for the management of enterocutaneous fistulas. A single intravenous infusion induced a response at four weeks in 50 to 81 percent of patients with refractory luminal disease and induced remission in 25 to 48 percent.¹ The response can be maintained with repeated infusions.² In patients with fistulizing disease, four weeks after the last of three infusions, at weeks 0, 2, and 6, 38 to 55 percent of fistulas had completely closed, and the median duration of response was 90 days.³ Clinicians often re-treat patients with infliximab after a relapse.

Infliximab therapy can result in the formation of antibodies against infliximab. The presence of these antibodies has been associated with infusion reactions in 6.9 to 19 percent of patients.⁴⁻⁹ These antibodies may also have shortened the duration of effect of repeated infliximab treatments. We studied the relation between antibodies against infliximab and postinfusion infliximab concentrations, the clinical effect of infliximab, and infusion-related side effects. In addition, we investigated risk factors for the formation of antibodies against infliximab.

METHODS

PATIENTS

We studied 125 consecutive patients with refractory luminal or fistulizing Crohn's disease who were starting treatment with infliximab between December 1998 and July 2000. All patients signed an informed-consent form describing procedures for safety follow-up. The study was approved by the institutional review board.

Patients were treated for active luminal disease with a single intravenous infusion of 5 mg of infliximab per kilogram of body weight and for draining fistulas with a series of three infusions of 5 mg per kilogram, at weeks 0, 2, and 6. If a patient had a response, therapy was repeated on relapse of the disease. The decision to re-treat was made by an experienced clinician when relapse was apparent as a result of increased diarrhea, increased abdominal pain, and decreased well-being. Infusions were administered under close supervision by a specially trained nurse at an infusion facility. An infusion reaction was defined as any significant adverse event

that occurred during the infusion or within two hours afterward.¹⁰ When an infusion reaction occurred, the infusion was stopped and restarted at a slower rate. If the symptoms recurred, 100 mg of intravenous hydrocortisone and 50 mg of intramuscular promethazine were given. This regimen was then given prophylactically 30 minutes before each subsequent infusion. Concomitant medications including immunosuppressive agents and corticosteroids were used as indicated to control bowel disease. Treatment with immunosuppressive agents consisted of azathioprine (2.0 to 2.5 mg per kilogram per day), mercaptopurine (1.0 to 1.25 mg per kilogram per day), or methotrexate (15 mg intramuscularly once weekly) for a median of 9.5 months (range, 2 to 84) before infliximab therapy began.

EVALUATIONS

All patients were evaluated before and every four weeks after each infusion, and side effects, including early reactions (infusion reactions) and late reactions (rash, arthralgia, fatigue, myalgia, and influenza-like symptoms), were recorded. Concentrations of infliximab and antibodies against infliximab in serum were measured at each visit and before each infusion. Each serum sample was assessed for infliximab and antibodies against infliximab in duplicate in a blinded fashion by Prometheus Laboratories; the values reported are the means. The infliximab assay was a microplate enzyme-linked immunosorbent assay in which infliximab bound to immobilized tumor necrosis factor α is detected with horseradish peroxidase-conjugated antihuman IgG (Fc-specific). The cutoff value, which was based on the mean (± 3 SD) value in serum samples from 40 patients who had never received infliximab, was 1.40 μ g per milliliter. Concentrations below the cutoff value are reported as negative. In the case of 140 samples, concentrations greater than 20 μ g per milliliter were reported as 21 μ g per milliliter.

The assay for antibodies against infliximab was a microplate enzyme-linked immunosorbent assay based on the double-antigen format in which infliximab is used both during the solid phase to capture antibodies against infliximab and during the biotinylated detection phase with Neutravidin-horseradish peroxidase. The value is reported in micrograms per milliliter on the basis of calibrations made with affinity-purified polyclonal rabbit antimouse IgG F(ab'). The mean cutoff value in serum samples from 40 patients who had never received infliximab

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was 1.69 μg per milliliter. Antibodies against infliximab were reported as negative when the concentration was less than 1.69 μg per milliliter and the serum infliximab concentration was less than 1.40 μg per milliliter and as indeterminate when the concentration was less than 1.69 μg per milliliter but the infliximab concentration was 1.40 μg per milliliter or greater. Because infliximab interferes with the assay, these values cannot be conclusively determined. The antibody test was considered to be positive when the concentration exceeded 1.69 μg per milliliter and the infliximab concentration was less than 1.40 μg per milliliter.

STATISTICAL ANALYSES

Patients were included in efficacy analyses if they had an initial clinical response and if they required further infusions for active luminal disease. Patients who underwent surgery and patients in whom the interval between infusions exceeded 20 weeks were excluded from the analysis. Patients with fistulas were included only if they required repeated infusions for active luminal disease after the initial three infusions. All serum samples with an indeterminate value for antibodies against infliximab were excluded from the analyses of antibodies against infliximab. The use of azathioprine, mercaptopurine, or methotrexate was considered as a single risk factor.

We used SAS software (version 6.12, SAS Institute) for all statistical analyses. Possible associations between two binary variables were assessed with use of the relative risk and corresponding 95 percent confidence interval. Analysis of variance was used for the analysis of continuous outcome variables with discrete explanatory variables. The Tukey multiple-comparison procedure was used to evaluate the statistical significance of a set of multiple comparisons. We used a generalized linear model to evaluate the simultaneous effect of two or more factors on the response variables. In some subgroups, we applied a nonparametric procedure (nonparametric one-way analysis), because of the small number of patients. The Kruskal-Wallis test was used to evaluate whether groups came from the same population. To investigate the relation between the duration of response and the concentration of antibodies against infliximab as well as infliximab concentrations, we used multiple linear regression analysis. The manufacturer of infliximab had no input into the design of the study, data analysis, or manuscript preparation. The analysis of the manufacturer's safety data was performed independently by the authors.

RESULTS

The characteristics of the 125 patients and their concomitant therapies are shown in Table 1. The median follow-up was 36 months (range, 25 to 48). Thirty-eight of the 125 patients (30 percent) received treatment for a fistula; and a total of 89 patients (71 percent) had a response to infliximab treatment.

ANTIBODIES AGAINST INFLIXIMAB

At base line no patient tested positive for antibodies against infliximab. After the fifth infusion, 76 patients (61 percent) had detectable antibodies (Fig. 1A). The incidence did not increase further with re-

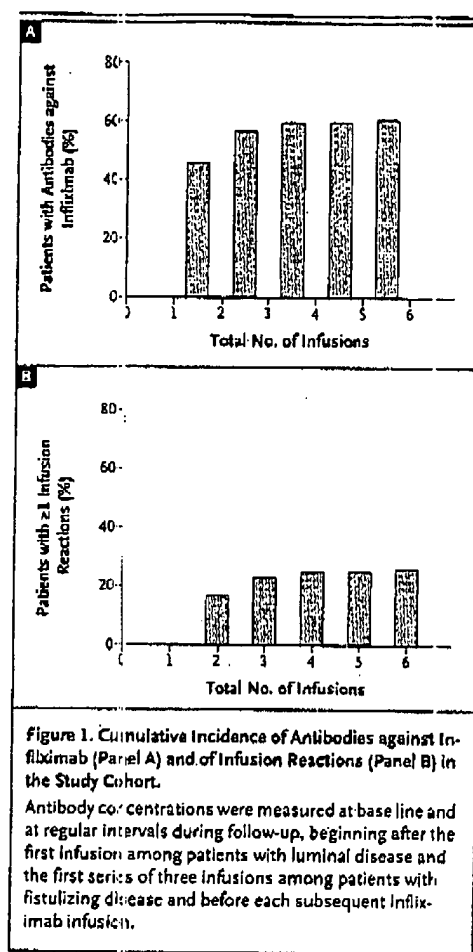
Table 1. Demographic Characteristics of the 125 Patients.

| Characteristic | Value |
|---|---------|
| Female sex — no. of patients (%) | 82 (66) |
| Age — yr | |
| Mean | 35 |
| Range | 17–73 |
| Type of disease — no. of patients (%) | |
| Ileitis | 34 (27) |
| Ileocolitis | 60 (48) |
| Colitis | 22 (18) |
| Ulcerative colitis-like | 6 (5) |
| Indeterminate | 3 (2) |
| Smoking status — no. of patients (%) | |
| Current | 43 (34) |
| Former | 9 (7) |
| Never | 49 (39) |
| Unknown | 24 (19) |
| Concomitant medications at entry — no. of patients (%) | |
| Corticosteroids | 53 (42) |
| Immunosuppressive agent: | |
| Azathioprine or mercaptopurine | 56 (45) |
| Methotrexate | 3 (2) |
| Mesalamine | 50 (40) |
| None | 18 (14) |
| Crohn's Disease Activity Index at first infusion* | |
| Mean | 260 |
| Range | 0–575 |
| C-reactive protein at first infusion — mg/liter | |
| Mean | 27.8 |
| Range | 0–163.4 |
| Active luminal disease at inclusion — no. of patients (%) | 87 (70) |
| Active draining fistulas — no. of patients (%) | 38 (30) |
| No. of infusions per patient† | |
| Mean | 3.9 |
| Range | 1–17 |

* Scores for the Crohn's Disease Activity Index can range from 0 to 600, with higher scores indicating more severe illness.

† Infusions were given over a mean period of 10 months.

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peated infusions. The serum concentrations of antibodies against infliximab had two clusters that could be separated with the use of 8.0 μg per milliliter as a cutoff value: 46 of the 125 patients (37 percent) had a titer of 8.0 μg per milliliter or greater. Patients who were taking immunosuppressive agents had a lower incidence of antibodies than patients who were not taking immunosuppressive agents: 43 percent (24 of 56), as compared with 75 percent (52 of 69) ($P < 0.01$). Immunosuppressive agents also protected against high titers of antibodies. Among patients taking immunosuppressive agents, as compared with those who were not taking immunosuppressive agents, the relative risk of antibody concentrations of 8.0 μg per milliliter or greater

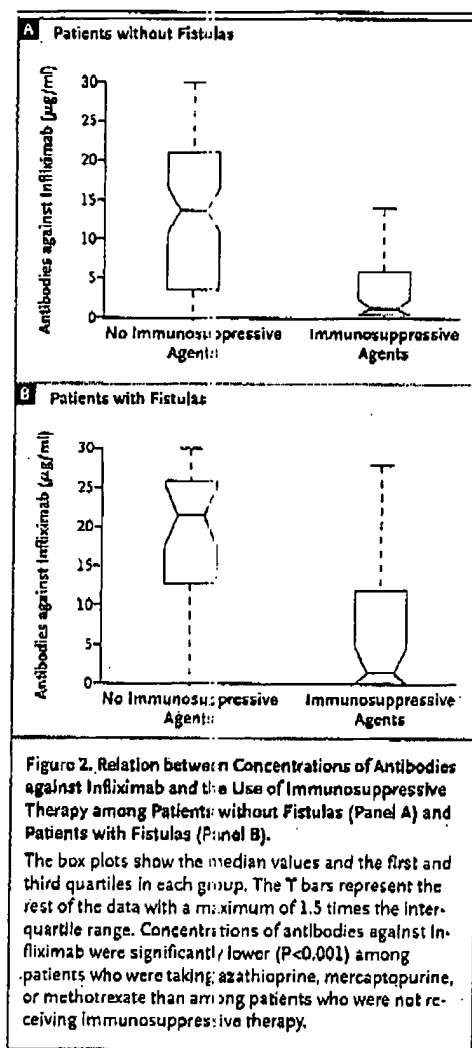
was 2.40 in the group without fistulas (95 percent confidence interval, 1.56 to 3.65; $P < 0.001$) and 2.85 in the group with fistulas (95 percent confidence interval, 1.54 to 5.25; $P < 0.001$). Among patients who were not taking immunosuppressive agents, the median concentration of infliximab antibodies was 13.8 μg per milliliter in the group without fistulas (95 percent confidence interval, 7.9 to 16.2) and 21.4 μg per milliliter in the group with fistulas (95 percent confidence interval, 13.2 to 24.5). Among patients who were taking immunosuppressive agents, the concentration of antibodies against infliximab was 1.3 μg per milliliter (95 percent confidence interval, 0.6 to 3.2) in the group without fistulas and 1.5 μg per milliliter (95 percent confidence interval, 0.4 to 8.8) in the group with fistulas ($P < 0.001$ by the Kruskal-Wallis test) (Fig. 2).

We found no association between antibodies against infliximab and sex, the location of disease, smoking status, or the use of mesalamine or corticosteroids at the time of an infliximab infusion. There was a weak positive relation between the three-infusion regimen (used at 0, 2, and 6 weeks in patients with a fistula) and the development of antibodies against infliximab ($P = 0.04$).

The cumulative incidence of infusion reactions was 27 percent. No reactions occurred during the first infusion, but the incidence increased during the subsequent infusions (Fig. 1B). There was a strong relation between the concentration of antibodies against infliximab and the occurrence of an infusion reaction. The median concentration was 20.1 μg per milliliter (95 percent confidence interval, 3.0 to 22.6) at the time of a first infusion reaction, as compared with 3.2 μg per milliliter (95 percent confidence interval, 1.6 to 4.9) among patients without an infusion reaction ($P < 0.001$) (Fig. 3A). Concentrations of 8 μg per milliliter or higher predicted a higher risk of infusion reactions (relative risk, 2.40; 95 percent confidence interval, 1.65 to 3.66; $P < 0.001$). Influenza-like reactions, arthralgia, rashes, fatigue, and myalgia were not related to the development of antibodies against infliximab.

We chose the time to the next infusion as a measure of the duration of response. There was a clear negative relation between the concentration of antibodies against infliximab and the duration of the response to infliximab ($P < 0.001$). Antibody concentrations of 8.0 μg per milliliter or greater were predictive of a shorter duration of response. The median duration of the response among patients with antibody concentrations below 8.0 μg per milliliter

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was 71 days (95 percent confidence interval, 57 to 88), as compared with 35 days (95 percent confidence interval, 28 to 42) among those with antibody concentrations of 8.0 µg per milliliter or more ($P < 0.001$) (Fig. 4).

INFLIXIMAB CONCENTRATIONS

A significant relation was found ($R^2 = 0.34$, $P < 0.001$) between the serum infliximab concentration at week 4 after an infusion and the concentration of antibodies against infliximab before that infusion. The overall median infliximab concentration four

weeks after an infusion was 12.0 µg per milliliter. As compared with patients who were not taking immunosuppressive agents, patients who were taking immunosuppressive agents were more likely to have infliximab concentrations of more than 12.0 µg per milliliter (relative risk, 1.33; 95 percent confidence interval, 1.40 to 2.60). In a logistic-regression analysis of variables that were predictors of an infliximab concentration of more than 12.0 µg per milliliter, including sex, type of disease, fistula treatment regimen, the number of infusions, and the use of corticosteroids, mesalazine, or immunosuppressive agents, only the use of immunosuppressive agents was significant ($P < 0.001$).

Infliximab concentrations four weeks after an infusion were significantly lower among patients with a first infusion reaction than among patients who had never had a reaction (1.2 µg per milliliter vs. 14.1 µg per milliliter, $P < 0.001$). Once patients had had an infusion reaction they received prophylaxis consisting of hydrocortisone and promethazine before subsequent infusions. Among patients who had no further reactions while receiving prophylaxis, concentrations stayed high at four weeks (median, 12.9 µg per milliliter; 95 percent confidence interval, 1.9 to 21.0). Infliximab concentrations, however, were almost undetectable (1.0 µg per milliliter; 95 percent confidence interval, 1.0 to 1.9; $P = 0.01$) among patients who had another reaction despite receiving prophylaxis (Fig. 3B). Once an infusion reaction occurred, the median duration of response to an infusion was shorter: 38.5 days (95 percent confidence interval, 34 to 51), as compared with 65 days (95 percent confidence interval, 56 to 71; $P < 0.001$). This shortened response persisted during further infusions irrespective of whether infusion reactions could be prevented with prophylaxis (median, 42 days; 95 percent confidence interval, 34 to 56) or not (median, 29 days; 95 percent confidence interval, 24 to 36; $P = 0.17$) (Fig. 3C).

The infliximab concentrations at four weeks were positively correlated with the duration of the response. Patients with infliximab concentrations of 12.0 µg per milliliter or greater had a median duration of response of 81.5 days (95 percent confidence interval, 68 to 98), as compared with 68.5 days (95 percent confidence interval, 52 to 77) among patients with infliximab concentrations of less than 12.0 µg per milliliter ($P < 0.01$). Logistic-regression analysis showed that the presence of antibodies against infliximab was independently associated with a shorter duration of response

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($P < 0.001$ by the Kruskal-Wallis test), whereas the use of immunosuppressive agents ($P = 0.58$) and the infliximab concentrations ($P = 0.70$) were not.

DISCUSSION

Infliximab has become common treatment for refractory Crohn's disease. Our data show that the development of antibodies against infliximab correlates with an increased risk of infusion reactions and with a shorter duration of response owing to lower infliximab concentrations. Our data suggest that treatment with immunosuppressive agents prevents infusion reactions and helps maintain clinical efficacy.

Several factors could account for the high incidence of antibodies against infliximab in this cohort of patients. Since serial measurements were obtained in patients who were treated intermittently, the longer intervals between treatments afforded more opportunities to measure antibodies without the confounding presence of infliximab in the serum. All patients in the Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy study of rheumatoid arthritis¹² and most patients in the ACCENT (A Crohn's Disease Clinical Trial Evaluating Infliximab in a New Long-Term Treatment Regimen) study of Crohn's disease⁴ received infliximab every eight weeks. Less than half the patients in our cohort were taking immunosuppressive agents, in contrast to the studies of rheumatoid arthritis, in which all patients were taking methotrexate maintenance therapy.¹⁰ The assays in

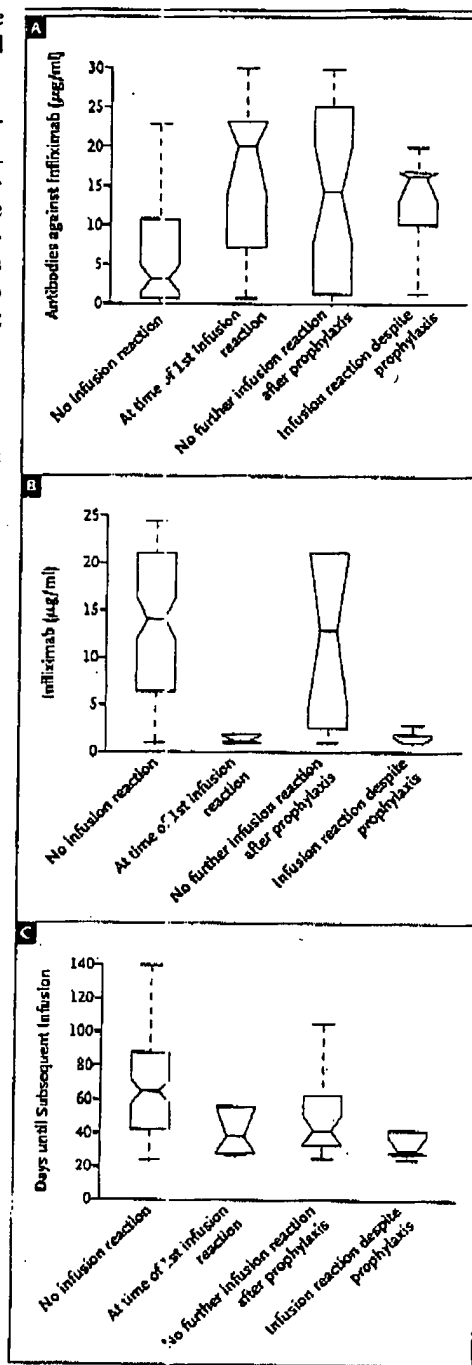


Figure 3. Concentrations of Antibodies against Infliximab Immediately before an Infliximab Infusion (Panel A), Infliximab Concentrations Four Weeks after the Infusion (Panel B), and the Duration of Response (Panel C), According to the Occurrence of Infusion Reactions.

Box plots show the median values and the first and third quartiles in each group. The T bars represent the rest of the data with a maximum of 1.5 times the interquartile range. The median antibody concentration in the group with no infusion reactions differed significantly ($P < 0.001$) from the median values in the three other groups. The median infliximab concentration in the group with no infusion reactions and the group with no further reactions after prophylactic therapy differed significantly ($P < 0.001$) from the median values in the other two groups. The median number of days until the subsequent infusion in the group with no infusion reactions differed significantly ($P < 0.001$) from the median values in the three other groups.

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our studies were performed at Prometheus Laboratories and differed from the assay used by Centocor.⁴ A formal comparison of the two assays is warranted, but it is unlikely that differences in methods will account for the differences in the incidence of antibody positivity.

The results of measurements of antibodies against infliximab in serum samples obtained between infliximab infusions are often indeterminate. We therefore investigated whether measurement of the infliximab concentration four weeks after an infusion provided more reliable results. Although there was a clear relation between infliximab concentrations and the duration of response, this correlation was not stronger than the correlation between the antibody concentrations and the duration of response.

A clear message from our study is that immunosuppressive treatment prevents the formation of antibodies against infliximab, thus reducing the incidence of infusion reactions and increasing the duration of response. Infusion reactions are important immunologic events induced by the presence of a substantial concentration of antibodies against infliximab in the serum. After an infusion reaction, infliximab disappears quickly from serum and is undetectable four weeks after an infusion. Once an infusion reaction occurred, the duration of the response to subsequent infusions decreased. Since antibodies develop soon after the initial infusion in most patients, we think immunosuppressive therapy should be instituted before infliximab therapy is started to prevent the formation of antibodies and improve the duration of response to the drug.

Whether any one of the immunosuppressive agents — azathioprine, mercaptopurine, or methotrexate — provides greater protection against immunogenicity remains to be determined. A drug interaction between methotrexate and infliximab has been proposed.¹² Although the maximal serum concentrations of infliximab are the same with or without concomitant methotrexate therapy, the rate of disappearance of infliximab was slower in one study among patients who were taking methotrexate than among those who were receiving infliximab alone.^{12,13} To overcome the immunogenicity prob-

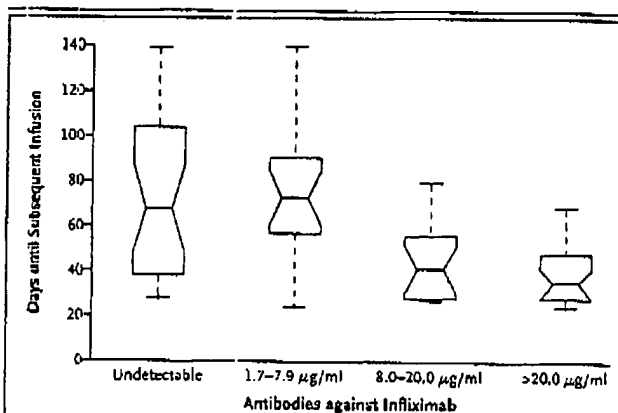


Figure 4. Duration of Response According to the Concentration of Antibodies against infliximab before an Infusion.

Box plots show the median values and the first and third quartiles in each group. The T bars represent the rest of the data with a maximum of 1.5 times the interquartile range. The four categories can be divided in two groups: the first two categories have concentrations of antibodies against infliximab of less than 8.0 µg per milliliter and the last two have concentrations of 8.0 µg per milliliter or greater. The median duration of response in the first two groups differed significantly ($P < 0.001$) from the median duration of response in the groups with titers of 8.0 µg per milliliter or higher.

lem, humanized and human antibodies have been engineered and studied in several diseases, including Crohn's disease.¹³⁻¹⁸ Further studies will show whether these antibodies are associated with a reduced incidence of immunogenicity and an increased ratio of efficacy to safety.

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Drs. Van Assche, D'Haens, and Rutgeerts have reported serving as consultants to Centocor and Schering-Plough. Drs. D'Haens and Rutgeerts have reported serving as paid speakers for Centocor and Schering-Plough. Dr. Van Assche has reported serving as a paid speaker for Centocor. Dr. Rutgeerts has reported receiving grant support from Centocor and Schering-Plough.

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